REMARKS

Claims 1, 2, 4, 6-9, 11, 15, and 16 are all the claims pending in the application. No amendment has been made.

Rejection under 35 U.S.C. § 103

Claims 1, 2, 4, 6-9, 11, and 15 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Ramirez *et al.* (previously cited) and Lamouse-Smith *et al.* (previously cited) in view of Martens *et al.* (previously cited), Swiss-Prot Accession No. P29460 (previously cited), Hatamoto *et al.* '(JP 09/009982) and Koonin *et al.*(previously cited).

Claim 16 stands objected to as being dependent from the rejected claim.

Ramirez and Lamouse-Smith are relied upon to teach that recombinant protein production in *E. coli* can be improved by increasing the available levels of amino acids present in the recombinant protein in levels substantially above the levels of that amino acid in E. *coli* proteins. The Office asserts that Lamouse-Smith specifically teaches that serine-family amino acids in particular are often present in higher levels in recombinant proteins and that the metabolic burden imposed by amino acid composition can be alleviated by supplementing the cell with required precursors. (Emphasis added.)

Martens is relied upon to teach the usefulness of IL-12 p40 as an IL12 antagonist for the treatment of septic shock and other conditions and the recombinant production of high levels of this protein in *E. coli.*

Swiss-Prot Accession No. P29460 is relied upon to teach the amino acid sequence of human IL-12 p40 showing that this protein has 3.3% cysteine residues.

Koonin is relied upon to teach the average amino acid composition of *E. coli* proteins (see Table 1) and specifically that on average *E. coil* proteins have only 1.1% cysteine residues.

Hamamoto is relied upon to teach methods of increasing the amount of cysteine produced in *E. coli* comprising transforming *E. coli* with a plasmid encoding the *E. coli* genes for *cysE, cysK* and pta and teach a vector encoding these genes.

The Office alleges that as Martens teaches that a skilled artisan would clearly desire to produce high levels of IL-12 p40 in *E. coli* and a comparison of Swiss-Prot Accession No. P29460 with the amino acid composition of *E. coli* proteins of Koonin shows that this protein has 3 times more cysteine than the average *E. coli* protein, it would have been obvious to produce the IL-12 p40 in an *E. coli* cell have increased levels of cysteine, such as the transformed cells of Hamamoto; and that one of skill in the art would have been motivated to use the cells of Hamamoto by the disclosures of Ramirez and Lamouse-Smith that recombinant protein production in *E. coli* can be improved by increasing the available levels of amino acids present in the recombinant protein in levels substantially above the levels of that amino acid in *E. coli* proteins.

Applicants respectfully disagree for the following reasons.

A. CysK expression does not supplement a precursor of serine-rich protein synthesis or serine synthesis

The Office states, in the second full paragraph, at page 3 of Office Action that "Lamouse-Smith et al. specifically teach that serine-family amino acids in particular are often present in higher levels in recombinant proteins and that the metabolic burden imposed by amino acid composition can be alleviated by supplementing the cell with required precursors."

Considering the fact that cystein is a product of cysteine synthase (CysK), it appears to Applicants that the Office considers cystein as a precursor of serine synthesis or serine-rich protein synthesis.

Applicants respectfully submit that the cystein synthase (CysK) enzyme does not produce a precursor of serine synthesis.

The enzyme cysteine synthase (CysK) catalyzes the chemical reaction:

O_3-acetyl-L-serine + hydrogen $\rightarrow$ sulfide L-cysteine + acetate. Therefore, the substrates of the CysK is acetyl-L-serine and hydrogen and the products of the cystein synthase are cystein and acetate.

Meantime, the synthesis of serine starts with the oxidatio of 3-phosphoglycerate forming 3-phosphohydroxypyruvate and NADN. Reductive amination of the ketone followed by hydrosis produces serine.

Accordingly, cystein is not a precursor of the biosynthesis of serine and, thus, the gene encoding cysK, when it is expressed in a recombinant host cell, does not supplement any precursor of serine synthesis or serine-rich protein synthesis.

B. None of References Teach the Introduction of cysK Gene in Serine-Rich Protein Production Systems

Applicant respectfully submit that all the cited references, single or in combination, fail to teach or disclose that introducing cysK gene induces high-yield production of serine-rich protein.

The Office alleges that Hamamoto teaches methods for increasing the amount of cysteine by introducing cysK. However, Hamamoto describes only the production of cysteine, not the increase in the production rate of serine-rich protein.

Neither do the cited references motivate one skilled in the art to use cysK gene in combined with a serine-rich protein coding gene in order to produce a serine-rich protein with reasonable expectation of success. Cysteine is not a precursor of serine synthesis, as discussed above, and there is no implication in any of the cited references that cystein or expression product of cysK, or even the presence of cysK gene in a recombinant host for producing a serine-rich protein will increase the serine-rich protein production and shorten the production time.

Furthermore, none of the cited references teach or suggests that a serine-rich protein also has a higher percentage of cystein level in the protein than the average *E. coli* protein. The Office states "Based on Lamouse-Smith, Swiss-Prot Accession No. P29460 and Koonin, because the IL-12 p40 has 3 times more cysteine than the average *E.coli* protein, it would have been obvious for one skilled in the art to increase the amount of cysteine by introducing cysK into *E.coli* in order to increase the amount of IL-12 p40 therein."

However, for the reasons discussed above, an increase in cysteine does not necessarily induce an increase in the production of a serine-rich protein. Also, nowhere in the cited references is indication of relationship between the cystein production or cystein amount in a host cell and the production of a serine-rich protein.

Even if it is true that the IL-12 p40 happens to have 3 times more cysteine than the average *E.coli* protein, there is no reason why one skilled in the art would have considered that all serine-rich proteins have more cysteine than the average *E.coli* protein. For example, when comparing Fig. 5a with Fig. 5b in the specification of the present invention, it can be seen that leptin, a serine-rich protein, has almost the same amount of cysteine as that of the average *E.coli* protein.

Therefore, there has been no motivation in the art at the time of the invention that one skilled in the art would have increased the level of cysteine in order to increase the amount of serine-rich proteins produced in a recombinant host. Also, none of the cited references motivate one skilled in the art to introduce cysK gene in order to increase the production of a serine-rich protein with a reasonable expectation of success.

Applicants found the production rate of a serine-rich protein could be increased by coexpression of a gene encoding the serine-rich protein and the cysK gene, which results in the currently claimed invention. The specification of the instant application shows that the claimed method allows to the leptin production up to 4 times faster upon the expression of both of leptin and cysK gene than the control, as well as greatly increases the productivity of leptin protein (Example 4). The currently claimed invention provides a superior advantage than the previous art, and the advantage is not suggested in the cited references.

Furthermore, Applicants respectfully draw the Office's attention to the fact that the amount of serine-rich proteins is not increased by amplifying cysE gene which is important to produce cysteine. In other words, the amplification of a gene involved in the production of cysteine does not necessarily induce or enhance the synthetic metabolism of a serine-rich protein. Therefore, one skilled in the art would not have conceived that the production rate of a serine-rich protein could be increased by coexpression of a gene encoding the serine-rich protein and the cysK gene.

Accordingly, it is believed that the rejection is not sustainable and its withdrawal is respectfully requested.

In the meantime, the Office states, in response to the Amendment filed on March 26, 2007, that "Applicants state that 'Lamouse teaches that when an amino acid is present in a recombinant protein at levels significantly higher than that present in host cellular proteins, the amino acid becomes a limiting factor in expression level. In other words, an increase in specific amino acid level does not directly induce an increase in specific protein production; rather, the production of specific amino acid is inhibited by feedback inhibition regulatory network.' However, the second sentence is not a recapitulation of the first in different words as suggested by applicants but instead a complete change of the meaning of the first statement. As such it is not clear what applicants are arguing."

Applicants hereby clarify the second statement pointed out by the Office as follows:

It means that although the amino acid level in a recombinant protein is significantly higher than that in host cellular proteins and the amino acid becomes a limiting factor in expression level, it does not always mean that the increase of the said amino acid level directly induces the increase of the said recombinant protein production. In particular, regarding cystein, one skilled in the art would not have expected that an increase in the level of cysteine induces an increase in the production of the recombinant protein because cysteine is regulated by the feedback inhibition regulatory network. Unlike amino acids, proteins are expressed by much more complex processes.

Also, the Office responds to Applicants' statement contained in the March 26, 2007 Amendment, by stating "Applicants argue that 'If cysteine increases in host cellular protein, cellular regulating systems will inhibit cysteine production and IL-12 p40 serine-rich protein production will not increase.' However, this is not persuasive as it misstates what would occur.

A correct statement of what would be expected is 'If the level of free cysteine increases intracellularly in the recombinant host, cellular regulating systems will inhibit cysteine production.' However, in view of the disclosures of Lamouse-Smith et al. and Ramirez et al., a skilled artisan would not expect the levels of free cysteine to be high at all, as all available cysteine would be expected to be incorporated into recombinant protein."

Applicants note that the above Office's statement is contrary to the disclosure of Hamamoto. Hamamoto uses a cysE variant for providing a feedback inhibition releasing type ([0080] paragraph in Hamamoto). In other words, Hamamoto uses a cysE variant for reducing the feedback inhibition because the cellular regulatory system inhibits the production of cysteine when cysteine is increased in host protein, not because all available cysteine would be expected to be incorporated into recombinant protein.

Therefore, because it has been known that the cysteine production is inhibited by a feedback inhibition system, one skilled in the art would not have expected that the production of recombinant protein having cysteine at a higher level would be increased by amplifying genes encoding an enzyme involved in cysteine synthesis. Moreover, there is no suggestion or teaching that one skilled in the art would have expected that amplifying genes encoding an enzyme involved in cysteine synthesis would induce an increase in the production of a serine-rich protein.

CONCLUSION

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the

Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

/Sunhee Lee/

Sunhee Lee
Registration No. 53,892

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE
23373
CUSTOMER NUMBER

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